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ABSTRACT

Saliva may be a useful alternative matrix for monitoring levofloxacin concentrations in multi-drug resistant TB patients. The objectives of this study were: a) to evaluate the correlation between plasma and salivary Lfx concentrations in MDR-TB patients; and b) to gauge the possibility of using saliva as an alternative sampling matrix for therapeutic drug monitoring of Lfx in TB endemic areas. This was a prospective pharmacokinetic study that enrolled MDR-TB patients receiving levofloxacin (Lfx; 750-1000mg once daily dosing) under standardized treatment regimen in Nepal. Paired blood and saliva samples were collected at steady state. Lfx concentrations were quantified using liquid chromatography- tandem mass spectrometry. Pharmacokinetic parameters were calculated using non-compartmental kinetics. Lfx drug exposure was evaluated in 23 MDR-TB patients. During the first month, the median (IQR) area under the concentration-time curve (AUC_{0-24}) was 67.09 (53.93-98.37) mg*h/L in saliva and 99.91 (76.80-129.70) mg*h/L in plasma, and the saliva plasma (S/P) ratio was 0.69 (0.53-0.99). Similarly, during the second month, the median (IQR) AUC_{0-24} was 75.63 (61.45-125.5) mg*h/L in saliva and 102.7 (84.46-131.9) mg*h/L in plasma with a S/P ratio of 0.73 (0.66-1.18). Furthermore, large inter-and intra-individual variabilities in Lfx concentrations were observed. This study could not demonstrate a strong correlation between plasma and saliva Lfx levels. Despite a good Lfx penetration in saliva, the variability in individual saliva-to-plasma ratios limits the use of saliva as a valid substitute for plasma. Nevertheless, saliva could be useful in semi-quantitatively predicting Lfx plasma levels.

INTRODUCTION

Levofloxacin (Lfx) belongs to the group A fluoroquinolones (FQ) for treating multi-drug resistant tuberculosis (MDR-TB), defined as resistance to at least isoniazid and rifampicin (1). This class of drug is used throughout the course of treatment in the new, shorter nine-month regimen, in the longer 24-month MDR-TB regimen and additionally in the six-month regimen for rifampicin susceptible, isoniazid mono-resistant TB(2). Lfx and moxifloxacin have been used inter-changeably in the longer regimen, however, in developing countries Lfx is preferred due to affordability, availability, better safety profile and fewer drug interactions with other medications(3, 4). Acquired FQs resistance during standard treatment resulting in poor outcomes shown in a prospective observational cohort study is of serious concern(5). An earlier study by the same group showed that 11.2% (79/832) of MDR-TB patients developed FQ resistance without any baseline resistance, potentially due to sub-therapeutic systemic concentrations of drugs achieved(6, 7). Similarly, other pharmacokinetic studies on Lfx in MDR-TB patients found considerable pharmacokinetic variability among individuals, with at least 25% of the patients not attaining desired plasma concentration and area under the concentration *vs* time curve (AUC_{0-24})(3, 4, 8, 9). It is clear that Lfx concentrations do not always reach the desired concentrations while administered in a standard dose. Therefore, measuring Lfx concentrations in plasma or other alternative matrices (saliva and dried blood spots) could help clinicians make informed dosing decisions. More so now, as the TB treatment marches towards individualization, therapeutic drug monitoring (TDM) using saliva sampling might become a game changer in TB treatment due to specific advantages over plasma sampling, in low resource settings(10, 11). The efficacy of Lfx is predicted by AUC_{0-24} and minimum inhibitory concentration (MIC)(12). Given as a monotherapy, the hollow fiber infection model on tuberculosis recently established a Lfx target of 146 for

maximum bacterial kill (EC_{80}) and 360 for the prevention of acquired drug resistance (12). Therefore, plasma AUC_{0-24} above 75 (if MIC is 0.5 mg/L) or 146 (if MIC is 1 mg/L) will be needed to attain the optimal target exposure for efficacy. With standard 750-1000 mg once daily dose, desired median peak concentration (C_{max}) was 8-13 mg/L while, median time to reach C_{max} (t_{max}) was 1-2h and median half-life ($t_{1/2}$) was 6-8h (13-15). Lfx demonstrated good penetration in extravascular body sites such as cerebrospinal fluid and cavitary lesions, due to rapid absorption and high volume of distribution(16, 17). Sasaki and Morishima compared Lfx levels in saliva and serum of eight healthy male volunteers after administration of 100 mg single dose. The study reported mean saliva/serum Lfx AUC ratio of 0.69 in fasting state and 0.56 in non-fasting state, indicating that saliva Lfx concentration could be useful for TDM(18). To date, however, studies comparing Lfx concentrations in plasma and saliva of MDR-TB patients have not been published. Saliva could be a useful alternative in predicting Lfx concentrations from plasma since sampling is non-invasive, fast, requires less rigid storage conditions, can be easily transported and is more suitable in children(19).

Therefore, the aims of this study were as follows: a) to evaluate the correlation between plasma and salivary Lfx concentrations in MDR-TB patients; and b) to gauge the possibility of using saliva as an alternative sampling matrix for TDM of Lfx in TB endemic areas.

PATIENTS AND METHODS

Patients and design

Study participants were MDR-TB patients undergoing treatment at German Nepal Tuberculosis Project (GENETUP), Nepal. This was a prospective pharmacokinetic study that

91 enrolled patients on treatment during 25/05/2016- 27/10/2017, with signed informed
92 consent. The study protocol was approved by Ethical Review Board of Nepal Health Research
93 Council, Kathmandu, Nepal (Reg. No 115/2016) and registered at clinicaltrials.gov (identifier
94 number NCT 03000517). Patients (≥18 years) with newly diagnosed or previously treated
95 MDR-TB (based on genotypic susceptibility testing to rifampicin by GeneXpert and culture)
96 receiving Lfx as a part of their MDR-TB regimen were eligible for inclusion. Subjects were
97 excluded if they had neurologic or severe extra-pulmonary manifestations of TB; had a body
98 weight <35kg, were on medications for the treatment of renal disorders, were breast feeding
99 or pregnant, were treated with aluminum- and magnesium containing antacids or ferrous
100 sulphate, cimetidine, probenecid, theophylline, warfarin, zidovudine, digoxin or cyclosporine
101 due to potential drug-drug interactions.

102 The national tuberculosis guidelines for the programmatic management of MDR-TB in Nepal
103 consists of an intensive phase of 8 months (with an addition of 4 months if there is no
104 culture/ conversion at the end of 6 months) followed by a continuation phase of 12 months
105 of treatment. Lfx (750-1000 mg once daily) was prescribed based on body weight as
106 described in the guidelines for management of drug resistant tuberculosis in Nepal. Other
107 drugs in this regimen included kanamycin (500-1000 mg/day i.m. injection), ethionamide
108 (500-750mg/day), pyrazinamide (20-30 mg/kg/day) and cycloserine (500-750 mg/day).

109 **Study procedures**

110 To assess Lfx concentrations, steady state blood and saliva samples were collected before
111 intake and at 1, 2, 4 and 8 hours after intake of Lfx. Patients were sampled twice i.e. at the
112 end of the first month (15-30th day) and second month (45-60th day) of treatment. Plasma
113 samples were collected in BD vacutainer vials (Becton, Dickinson and Company, NJ, USA,

114 catalog no. 23-021-016) whereas, saliva samples were collected using two different
115 techniques. Saliva samples were collected using a salivette® (Sarstedt, Nümbrecht, Germany,
116 catalog no. 50-809-199) and additionally filtered using a membrane filter (0.2µm diameter,
117 Merck KGaA, Darmstadt, Germany)(20). The collected plasma/saliva samples were
118 centrifuged and frozen at -30°C until analysis. A standardized data collection (case report
119 forms and excel database file) was created to record demographic and clinical data of the
120 included patients. HIV test was carried out for all included patients as a part of treatment
121 protocol, but none of the included patients were HIV positive.

122 **Drug quantification in plasma and saliva**

123 Lfx concentrations in human plasma and saliva were analyzed in the laboratory of the
124 department of Clinical Pharmacy and Pharmacology at the University Medical Center
125 Groningen, Netherlands using a validated liquid-chromatography tandem mass spectrometry
126 technique (LC-MS/MS)(21). The calibration curve was linear over a range of 0.10-5 mg/L for
127 Lfx. To encompass concentrations levels above 5 mg/L, dilution integrity was determined to
128 accurately measure concentrations levels up to 40mg/L.

129 The pH of salivary samples was measured using a pH indicator strips (Merck KGaA,
130 Darmstadt, Germany), encompassing the pH range from 2.0-9.0, with 0.5 pH units increment
131 distinguished by color change. Two independent observers (S.G., SHJ.VDE.) recorded the
132 results, and in case of differences consensus was reached in the presence of a third observer
133 (A.GM.).

134 **Data analysis**

PK analysis. For PK parameters, non-compartmental analysis was performed using MW/Pharm (version 3.82; Mediware, Groningen, the Netherlands). The PK parameters included: maximal plasma concentration (C_{max}), corresponding time of C_{max} (t_{max}), area under plasma concentration vs. time curve (AUC_{0-24}), apparent volume of distribution (Vd/F), apparent clearance (CL/F), half-life ($t_{1/2}$) and elimination constant for plasma and saliva (k_e). Statistical analysis was performed using SPSS Inc. (v 23.0, Chicago IL, USA). Results are presented as medians with interquartile range (IQR) for continuous variables and number percentage (%) for categorical variables. The normal distribution of data was ascertained by skewness-kurtosis, visual inspection of boxplots and Shapiro-Wilk test. The non-parametric Wilcoxon signed rank test was used to assess the differences between plasma and saliva PK parameters, when applicable. Inter- and intra- individual pharmacokinetic variabilities were evaluated from the CV% calculated as the quotient of standard deviation divided by the mean plasma concentration multiplied by 100. Passing-Bablok regression was used to assess the relationship between saliva and plasma Lfx concentrations (R Statistical Software). All P values were reported as significant if $P < 0.05$.

RESULTS

Study subjects. Twenty-three MDR-TB patients were included in the study and demographic and baseline clinical characteristics are shown in Table 1. In our study, 70% (16/23) were male. The median age was 32 (*IQR* 28-47 years), body weight was 48 (*IQR* 41-55 kg) with a body-mass index (BMI) of 18 (*IQR* 16-19 kg/m^2). Based on BMI, 65% (15/23) of the patients were underweight, as a result once daily 750-1000 mg Lfx dosing resulted in mg/kg doses of 17.14 (15.38-19.23). All 23 (100%) patients completed the first PK sampling (15-30th day). However, during the second month, 4 (13.1%) patients failed to participate in PK sampling.

158 One patient was transferred out, while two-patients were shifted to pre-XDR category,
159 whereas the remaining patient participated only in saliva sampling.

160 **Lfx PK.**

161 The steady-state Lfx PK parameters are mentioned in Table 2. During the first month, the
162 median area under the concentration-time curve (AUC_{0-24}) was 67.09 (*IQR* 53.93-98.37)
163 mg*h/L in saliva and 99.91 (*IQR* 76.80-129.70) mg*h/L in plasma, and the saliva plasma (S/P)
164 ratio was 0.69 (*IQR* 0.53-0.99). Moreover, the median C_{max} was 7.03 (*IQR* 5.61-9.02) mg/L in
165 saliva and 10.35 (9.10-11.44) mg/L in plasma with the S/P ratio of 0.68 (*IQR* 0.53-0.97). A
166 moderate positive correlation (r_s=0.50; p=0.016) was demonstrated between the saliva and
167 plasma AUC_{0-24}. Similarly, during the second month, the median AUC_{0-24} and C_{max} were 75.63
168 (*IQR* 61.45-125.5) mg*h/L and 8.30 (*IQR* 6.56-12.03) mg/L in saliva and 102.7 (*IQR* 84.46-
169 131.9) mg*h/L and 10.96 (*IQR* 9.34-11.58) mg/L in plasma. The median AUC_{0-24} S/P ratio was
170 0.734 (*IQR* 0.66-1.18). This time, saliva and plasma AUC_{0-24} showed a strong positive linear
171 relationship (r_s =0.754; p=0.0001) compared to the first month. Assuming a Lfx plasma
172 protein binding of 24%, the median free plasma *f*AUC_{0-24} was 75.93 (58.37-98.57 *IQR*)
173 mg*h/L in the first month and 78.05 (64.19-100.24 IQR) mg*h/L in the second month of
174 treatment. The median S/P ratios were 0.96 (0.95-1.25 *IQR*) and 0.88 (0.92-0.99 *IQR*)
175 respectively. The unbound Lfx *f*AUC_{0-24} in plasma reflected its salivary AUC_{0-24} closely, with
176 S/P ratio almost close to 1. Lfx concentration-time curves for both plasma and saliva are
177 shown in Figure 1.

178 Furthermore, a trend towards moderately positive correlation (r_s=0.379; p=0.021) was
179 observed when Lfx C_{min} in saliva was evaluated to predict its AUC_{0-24} in plasma (r= 0.38;

estimated linear regression equation). When saliva C_{min} was below 2 mg/L, proportion of patients had plasma AUC_{0-24} below desired 146 (12) given MIC was at 1 mg/L.

Passing Bablok regression analysis was used to evaluate the agreement between plasma and saliva Lfx concentrations. Figure 2 shows fitted Passing-Bablok regression that revealed a linear relationship and was close to the line of identity (x=y) with an estimated slope 95% CI of 1 (-2.11 to 2.57) for first month and 1.81 (-0.51 to 3.92) for second month. Similarly, the intercept was -1.85 (-9.81 to 16.42) and -7.17 (-21.26 to 0.95) respectively. In both months, 95% CI range included 1 for slope and 0 value for intercept, thereby satisfying the condition for line of identity.

The inter-individual variability was assessed in 208 Lfx measurements in plasma and 195 measurements in saliva at 0, 1, 2, 4, and 8 h samples. We found large inter-individual variability in Lfx concentrations. Furthermore, intra-individual variability was evaluated for the same patient based on the Lfx concentrations in plasma and saliva, between first and second months of treatment. The median intra-individual variability CV_{intra} was 8.77 (*IQR* 3.56-24.90 %) in plasma and 24.25 (*IQR* 12.20-34.65 %) in saliva for (19/23) patients. In our study, the intra-individual variability was lower than inter-individual variability. Table 3 shows inter-and intra-individual coefficients of variation for Lfx. The salivary pH ranged from 4.5-8.0 for different individuals with a mean value of 5.78 in the first month and 5.96 in the second month. Lfx saliva-plasma ratio as a function of salivary pH are plotted together (Figure 3).

DISCUSSION

The presence of Lfx in MDR-TB regimen has been associated with greater treatment success and reduced death(22). Despite this dominant position as a 2nd line TB drug, many clinical

trials have shown inadequate Lfx concentrations in plasma of MDR-TB patients that has refrained the drug from achieving its maximum efficacy(4, 8, 9). The measurement of drug concentrations in plasma of MDR-TB patients and dose adjustments thereafter have contributed positively to MDR-TB treatment outcomes(23). Yet, only few TB treatment centers worldwide have adopted TDM. Officially, the importance of TDM in the management of patient's sub-groups of drug-susceptible tuberculosis was first introduced in the clinical practical guidelines by the American Thoracic Society, Centers for Disease Control and Prevention and, Infectious Diseases Society of America and was endorsed by the European Respiratory Society and the US National Tuberculosis Controllers association(24). Among many logistic and financial challenges that have hindered TDM implementation, one problem is that venous sampling does not always have enough leverage in low-resource TB endemic settings, mainly due to the invasive nature of sampling, need of skilled personnel for venipuncture, potential infectious hazard, cooling requirements for transportation and storage, and high costs (11). In this scenario, use of alternative and stress-free sampling matrixes such as saliva could imprint TDM strategy in the national TB treatment guidelines. Therefore, in this first study on salivary penetration of Lfx in MDR-TB patients, we evaluated saliva's potential as an alternative sampling matrix and to explore whether it can quantitatively reflect plasma concentrations for TDM guided dosing. Overall, the salivary and plasma concentration-time profiles agreed well for different patients characterized by higher Lfx concentrations in plasma than in saliva except in 21% (5/23) of patients who had higher salivary concentrations. The amount of Lfx present in saliva is representative of its free fraction in plasma that is able to passively diffuse to saliva, which happens almost instantaneously due to a concentration gradient(25). Given Lfx's variable degree of protein binding (24-40%) in different individuals, we found large inter-individual variation in salivary

concentrations[22]. The results obtained from plasma samples were more homogenous and consistent with recently published studies on MDR-TB patients by van't Boveneind-Vrubleuskaya et al. and Peloquin et al. with similar median observed AUC_{0-24} and C_{max} values(4, 15). In theory, several factors could explain the high inter-individual variability in saliva, such as salivary pH in combination with drug pKa, salivary flow rate, and mechanism of drug transport (passive or active)(25, 26). The degree of ionization in different compartments is generally explained by pH of the compartments and the pKa of the drug. For example, lipid soluble non-ionized drugs which are not extensively bound to plasma proteins can easily transfer across the phospholipid bilayer of salivary cell membranes compared to ionized hydrophilic ones which tend to retain in plasma(26, 27). The pKa values for Lfx are 5.35 (strongest acidic) and 6.72 (strongest basic) and a saliva pH range was 4.5-8 [25]. In patients with high salivary Lfx levels, it could be hypothesized that higher salivary concentrations could be the function of acidic salivary pH and basic drug pKa values that permitted swift transfer of Lfx from plasma to saliva and ionization thereafter. However, due to the unavailability of actual drug pKa data and unbound Lfx fraction in plasma for individual patients, we couldn't attribute salivary pH alone to explain the variabilities in salivary Lfx concentrations. In addition, patient hydration state is thought to influence parotid salivary flow rates and in turn saliva derived drug concentrations. As saliva mainly constitutes water (97-99.5%) originating from plasma by acinar cells, it is hypothesized that decrease in water volume due to dehydration would result in loss of salivary production (28). Fischer and Ship reported that dehydration significantly decreases the salivary output (29) but could not establish a strong correlation between biological markers of hydration (haematocrit, haemoglobin, serum sodium, plasma protein, creatinine, serum and urine osmolality) and salivary output, in their study (30). Therefore, influence of

hydration/dehydration status on salivary Lfx concentrations needs to be studied. Furthermore, presence of active transport channels might have contributed to high salivary concentrations, which have been studied for some peptides like insulin but not for Lfx yet and needs to be validated(27).

A noteworthy finding of our study was that Lfx in saliva does not accurately predict its plasma levels, due to variable S/P ratios at different months of treatment and large inter-individual variability in Lfx saliva concentrations CV% (min, max) of 44.90% and 94.29%. Furthermore during the second month of treatment, high inter-individual variabilities were observed at mean t4 concentrations in both matrixes (Figure 1), cause of which could not be identified since the clinical study procedures were uniform and patients were on the same regimen for at least first two months of treatment.

This observation is not surprising as anti-TB drugs (levofloxacin, moxifloxacin, isoniazid, rifampicin and linezolid among others) exhibit high rates of PK variability even in plasma. Moreover, alternative matrices for TDM such as dried blood spots and saliva rarely have the level of precision that plasma based assessment possess. Despite the limitations, the potential utility of saliva in semi-quantitative testing remains strong. Patients with Lfx C_{min} below 2 mg/L in saliva were at the risk of lower plasma AUC_{0-24} (Figure 4). These patients are likely to benefit from semi-quantitative saliva based TDM in resource limited settings. However, this simple and non-invasive saliva based TDM may present few a challenges. First, the sampling procedure using salivette introduces variability in recovery depending on the type of cotton rolls used (plain cotton swab, cotton swab impregnated with citric acid, and synthetic cotton swab). We found that around 30% of Lfx was sorbed to plain cotton rolls used for collection of saliva samples. Therefore, the saliva sample collection procedure

274 should be standardized and well-controlled. The salivette technique further requires
275 centrifugation for recovery of collected saliva. Alternatively, saliva samples could be
276 collected by compressing the cotton roll in a syringe equipped with membrane filter (20).
277 Second, it will not be feasible to analyze Lfx levels in saliva by advanced LC-MS/MS in
278 resource limited settings. It has been prior suggested that patients at risk of low FQ exposure
279 can be distinguished from those with normal/high exposure by a semi-quantitative point of
280 care test such as spectrophotometer/thin-layer chromatography at a local level (31). The
281 early pre-selection using semi-quantitative testing will act as a gate-keeper, only selecting
282 patients at risk to offer TDM with expensive high-performance liquid chromatography
283 technique at regional level, thereby optimally allocating resources from already depleted TB
284 programs (10, 31). Therefore, development of a simplified, affordable, point-of care tool for
285 determination of Lfx levels in saliva should be a priority. Third, thermal instability of anti-TB
286 drugs and the need of refrigeration and cooling conditions for transportation might be an
287 issue. We recently investigated the impact of high temperature exposure on stability of Lfx
288 and found that it was stable at 50°C for 10 days. This is advantageous, as it precludes the
289 cooling requirements for transportation of samples to the laboratories for TDM. Preferably,
290 in remote settings, dried blood spots sampling could be a feasible option due to longer
291 stability at room temperature and transportation option by regular mail but still requires the
292 advanced LC/MS-MS for analysis. Another attraction in the field of alternative sampling
293 could be dried saliva spots but requires sensitive high cost equipment, analytical method
294 development and validation, and long term stability testing at higher temperatures.

295 In this study, we could not use the Bland Altman method for graphical representation of
296 mean and 95% (SD) limits of agreement between Lfx concentrations in plasma and saliva.
297 The one sample t-test showed that the log differences between saliva and plasma

concentrations were significantly different ($p < 0.05$) from 0, which violates one of the assumptions of Bland-Altman analysis.

There were some limitations in our study. First, the sample size of 23 was rather small to explain the observed high Lfx inter- and intra- patient variability in saliva compared to plasma. To explain this effect in saliva, studies with sample size that ensures statistical power of more than 80% will be needed. Second, different predictors of salivary Lfx concentrations such as salivary flow rate were not studied. Despite the limitations, salivary Lfx concentrations could contribute as a valuable semi-quantitative pre-selection tool to identify patients' sub-groups eligible for TDM using dried-blood spot. Patients with Lfx C_{min} below 2 mg/L in saliva could benefit from TDM due to the risk of lower plasma AUC_{0-24}.

In conclusion, this study could not demonstrate any significant relationship between plasma and saliva Lfx levels. Although Lfx penetrated in saliva, the variability in individual saliva-to-plasma ratios limits the use of saliva as a valid substitute for plasma. Despite the limitations, our data suggest that the potential utility of saliva in semi-quantitative testing remains strong. Patients with Lfx C_{min} below 2 mg/L in saliva are likely to benefit from semi-quantitative saliva based TDM in resource limited settings.

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429 **Table 1: Baseline characteristics of all included patients**

Patient Characteristics	Value
Demographic (n=23)	
Male	16 (69.56)
Age, years	32 (28-47)
Body weight, kg	48 (41-55)
Length, cm	165 (162-175)
BMI (kg/m^2)	17.96 (16.23-18.83)
Underweight (<18.5 kg/m^2)	15 (65.22)
Normal (18.5-25.0 kg/m^2)	8 (34.78)
Co-morbidities	
Diabetes mellitus	2 (8.69)
HIV	0
Dose (mg/kg)	
Lfx	17.14 (15.38-19.23)
Renal function, baseline	
Creatinine, µmol/L	70.72 (61.88-79.56)
Urea, mg/dl	19 (15-23)
Sodium, mmol/L	140 (134-144)
Potassium, mmol/L	4.12 (3.83- 4.4)

430 Data are presented as n (%) or median (interquartile range)

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435 **Table 2: Steady-state pharmacokinetic parameters for Lfx**

Parameters	Plasma		Saliva		S/P ratio	S/P ratio	p-value (plasma)	p-value (saliva)
month	I (n=23)	II (n=19)	I (n=23)	II (n=20)	I	II	I and II	I and II
AUC_{0-24}, mg*h/L	99.91 (76.80-129.70)	102.7 (84.46-131.90)	67.09 (53.93-98.37)	75.63 (61.45-125.5)	0.69 (0.53-0.99)	0.74 (0.59-0.93)	1.00	0.05
***f*AUC_{0-24}, mg*h/L**	75.93 (58.37-98.57)	78.05 (64.19-100.24)	-	-	0.88 (0.92-0.99)	0.96 (0.95-1.25)	0.17	-
C_{max}, mg/L	10.35 (9.10-11.44)	10.96 (9.34-11.58)	7.03 (5.61-9.02)	8.30 (6.56-12.03)	0.68 (0.53-0.97)	0.73 (0.66-1.18)	0.72	0.07
t_{max}, h	2 (1.08-4)	2 (1-2.06)	2 (1.3-4)	2 (1.04-3.36)	-	-	0.34	0.23
CL/F, L/h	6.75 (4.72-9.46)	7.94 (5.09-9.34)	9.58 (6.74-12.33)	8.99 (5.92-11.80)	-	-	0.93	0.52
Vd/F, L	87.9 (72.54-106.40)	88.84 (55.73-101.2)	124.3 (111.45-157.30)	125.60 (83.04-158.25)	-	-	0.13	0.87
$t_{1/2e}$	8.77 (6.50-10.71)	7.86 (6.32-10.11)	8.58 (7.97-10.36)	8.47 (6.23-14.02)	-	-	0.94	0.96
K (/h)	0.08	0.08	0.10	0.08	-	-	0.94	0.96

	(0.06-0.11)	(0.07-0.08)	(0.07-0.12)	(0.05-0.11)				

Data are presented as median (interquartile range). AUC_{0-24}, area under the concentration-time curve; ***f*AUC$_{0-24}$**, free Lfx **AUC$_{0-24}$** assuming plasma protein binding of 24%; C_{max}, maximum concentration of drug; t_{max}, time at which C_{max} occurred; CL/F, apparent total body clearance; Vd/F, apparent volume of distribution; $t_{1/2e}$, elimination half-life; k, elimination rate constant.

Table 3: Inter-individual (CV_{inter}) and intra-individual (CV_{intra}) variabilities of Lfx

Inter-individual variability (n=23)	Plasma concentration, mean (SD); (CV_{inter}%)		Saliva concentration, mean (SD);(CV_{inter}%)	
Time (h)	I month	II month	I month	II month
0	1.70, 1.14; 67.29	1.63, 1.06; 65.39	1.32, 1.02; 77.02	1.70, 1.60; 94.29
1	8.26, 2.47; 41.98	7.23, 3.65; 50.43	5.58, 2.88; 51.58	5.63, 4.34; 77.02
2	8.42, 2.36; 27.98	9.91, 1.90; 19.13	5.56, 2.70; 48.53	8.46, 3.80; 44.90
4	7.61, 2.04; 25.85	7.84, 1.92; 24.43	5.09, 3.10; 61.02	6.53, 5.28; 80.80
8	6.40, 3.72; 58.11	6.14, 3.37; 54.89	4.05, 2.31; 56.91	4.85, 3.01; 61.97
Intra-Individual variability, CV_{intra} %	8.77 (3.65-24.90) * (n=19)		24.25 (12.20-34.65) * (n=20)	

*= Median (interquartile range). SD, standard deviation; CV%, co-efficient of variation.

Legends for Figures.

Figure 1: Lfx plasma and saliva concentration-time curves (mean ± SEM)

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445 Figure 2: Passing-Bablok regression analysis of mean Lfx concentrations (t0, 1, 2, 4, 8 h) in
446 plasma and saliva for two months. The bold solid line represents the Passing-Bablok fitted
447 line, whereas the solid line is the line of identity. The dashed lines are 95% CI; r is the
448 spearman's rank co-relation; and N is the number of paired mean plasma and saliva
449 concentrations.

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451 Figure 3: Lfx saliva-plasma ratios at different time-points (0, 1, 2, 4, 8h) and salivary pH at
452 first and second month of treatment.

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454 Figure 4: Lfx C_{min} in saliva to predict plasma AUC_{0-24}. The vertical line at 2 mg/L is the C_{min} cut-
455 off.

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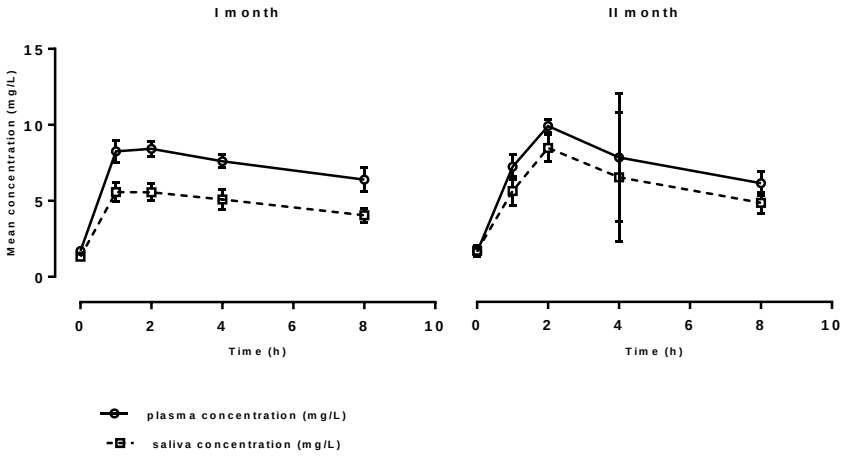

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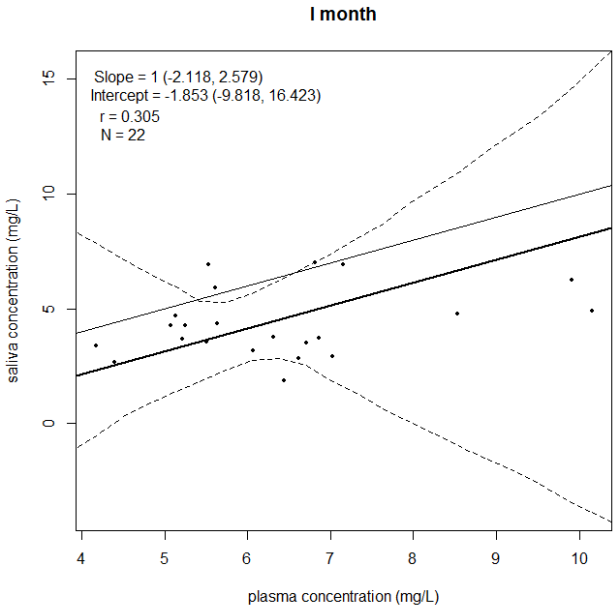

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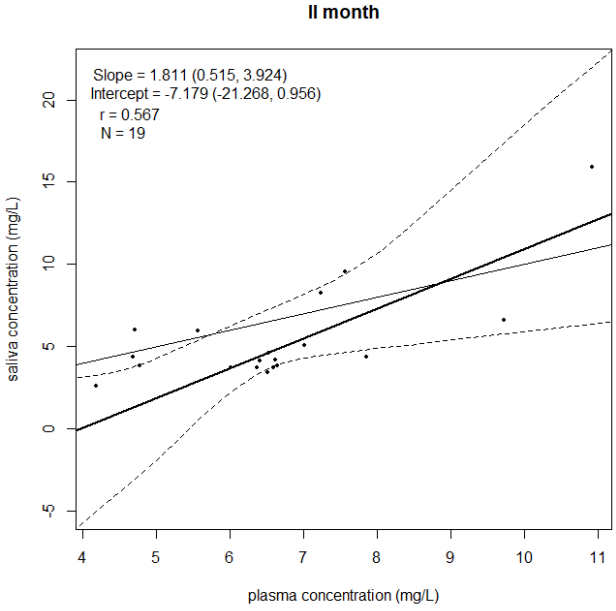

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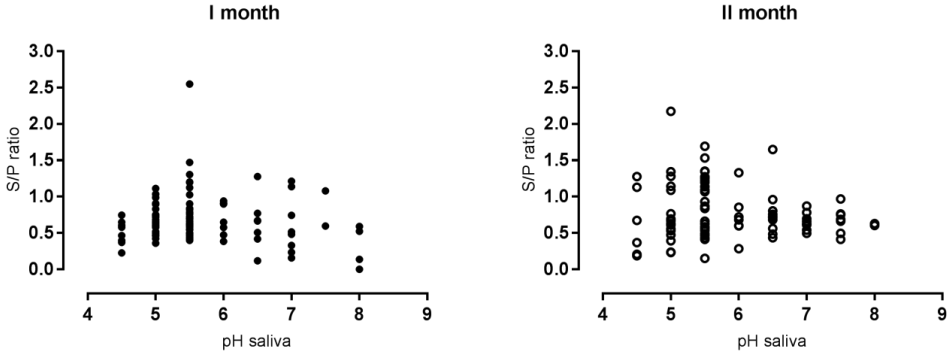

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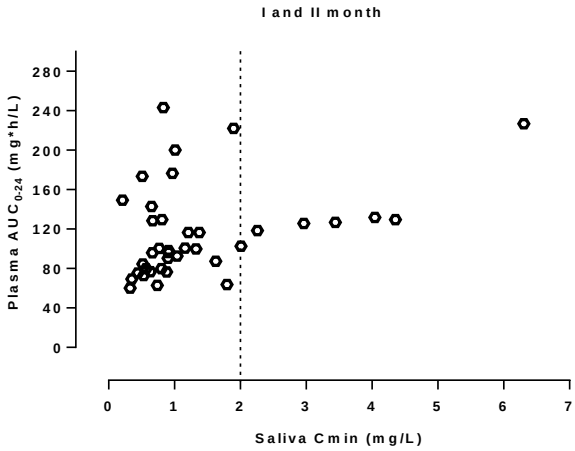

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